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REPORT OF THE ADVISORY COMMITTEE ON 2,4,5-T TO THE ADMINISTRATOR OF THE ENVIRONMENTAL PROTECTION AGENCY

ENVIRONMENTAL PROTECTION AGENCY

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ADVISORY COMMITTEE ON 2,4,5-T

to

THE ADMINISTRATOR

of the

ENVIRONMENTAL PROTECTION AGENCY

Submitted, May 7, 1971

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CHILDREN'S HOSPITAL MEDICAL CENTER

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May 7, 1971

Mr. William D. Ruckelshaus
Administrator
Environmental Protection Agency
Washington, D.C. 20460

Dear Mr. Ruckelshaus:

On behalf of the membership of the Advisory Committee on 2,4,5-T, I am pleased to submit the attached Report.

We earnestly hope the Report will prove helpful to you and your staff in establishing policy and procedures relative to future regulation of the use of the herbicide 2,4,5-T.

I shall endeavor to be of further service in this connection if needed.

Sincerely yours,

James G. Wilson

James G. Wilson, Chairman
Advisory Committee on 2,4,5-T

JGW:bh

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INTRODUCTION

On October 29, 1969, the President's Science Advisor, Dr. Lee A. DuBridge, announced that a series of coordinated actions was being taken by several governmental agencies to restrict the use of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). This was precipitated by release a few days earlier of the findings of a large-scale screening study of a number of pesticides and industrial chemicals conducted by Bionetics Research Laboratories in which it was found that mice and rats treated during early pregnancy with large doses of 2,4,5-T gave birth to defective offspring.

The announcement, together with reports of an increased occurrence of birth defects by South Vietnamese newspapers during June and July 1969, elicited far-reaching reactions from governmental agencies, segments of the scientific community, various lay groups concerned with environmental problems, and from the public communications media. Government-sponsored panels of experts, special commissions set up by scientific organizations, hearings before subcommittees of the U.S. Senate, and conferences attended by representatives from industry, government, and universities examined available data and heard expert opinions. None of these groups, however, was able to provide a generally acceptable answer to the central question of whether 2,4,5-T, as currently produced and used, constituted a risk for human pregnancy. At least one reason for failure to reach a satisfactory resolution of the issue was the paucity of reliable, scientific evidence.

Additional animal experiments performed early in 1970 confirmed that the purest available sample of 2,4,5-T, given in large doses to

pregnant mice, did indeed result in the delivery of some malformed offspring. The question then becomes one of whether, or to what extent, such animal data could be extrapolated to man. On April 14, 1970, an attitude of caution was expressed by the Secretary of Health, Education and Welfare, who advised the Secretary of Agriculture that: "In spite of these uncertainties, the Surgeon General feels that a prudent course of action must be based on the decision that exposure to this herbicide may present an imminent hazard to women of child-bearing age." Accordingly, on the following day the Secretaries of Agriculture, of Health, Education, and Welfare and of Interior jointly announced the suspension of the registration of 2,4,5-T for: "I. All uses in lakes, ponds or on ditch banks. II. Liquid formulations for use around the home, recreation areas and similar sites." (USDA-PRD PR 70-1, 20 Apr. 1970) A notice for cancellation of registration was issued on May 1 for: "I. All granular 2,4,5-T formulations for use around the home, recreation areas and similar sites. II. All 2,4,5-T uses on crops intended for human consumption." (USDA-PRD PR 70-13, 1 May 1970) All registrants of 2,4,5-T were advised of these actions, and two of the registrants, Dow Chemical Company and Hercules Incorporated, exercised their right under Section 4.c. of the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 135 et seq.) to petition for referral of the matter to an Advisory Committee.

The National Academy of Sciences supplied a list from which was selected a nine-member Advisory Committee of scientists with appropriate qualifications from universities and research institutes over the country. At its first meeting on February 1 and 2, 1971, the Advisory Committee was given a charge which in substance asked that it: 1) consider all

relevant facts, 2) submit a report and recommendations regarding registration for certain uses of 2,4,5-T, and 3) state the reasons or bases for these recommendations. It was the concensus of the Committee that the central issue was whether use of the herbicide does in fact constitute an imminent health hazard, especially with respect to human reproduction. Accordingly, the Committee has undertaken to examine all available information and to evaluate its relevance to the potential hazard of human exposure during pregnancy.

During the intervening months since restrictions were placed on the use of 2,4,5-T a number of additional studies have been carried out on several animal species and a few reports on human exposure during pregnancy have been further evaluated. Although the new data have not answered all of the questions that have been or could be raised, they have undoubtedly provided a more substantial basis for making a scientific judgment about possible effects of this herbicide on prenatal development than previously existed. In undertaking such judgment the Committee has taken into account certain considerations that seem appropriate to the issue, as follows: 1) As is frequently the case, available data are insufficient for a definitive statement of conditions under which a specified risk might occur, assuming that freedom from risk is ever attainable. 2) Since most chemicals under suitable laboratory conditions could probably be demonstrated to have teratogenic effects, and certainly all could be shown to produce some toxic effects if dosage were raised high enough, it would not be reasonable to consider the demonstration of toxic effects under conditions of greatly elevated dosage sufficient grounds for prohibiting further use of a particular chemical. 3) Benefits are to be expected from the continued use of

2,4,5-T. The necessity of making a value judgment of benefit vs. risk, therefore, must be accepted, not only for this herbicide, but for numerous valuable drugs, some natural nutrients, and many other chemicals, some of which are known to be teratogenic in laboratory animals. The risk vs. benefit judgment for a particular herbicide or drug can be evaded only if it can be shown that another compound is equally as efficient and involves less risk. This presupposes that the risk potential of a substitute herbicide is at least as well known as that of the original (in this case 2,4,5-T), a fact that may be difficult or impossible to ascertain. The substitution of a relatively unknown pesticide for an older one with known adverse effects is not a step to be taken lightly. Even with steadily improving methods for safety evaluation of new chemicals it is impossible to anticipate all of the conditions and permutations of use that could result in undesirable effects.

The task of making a judgment about the central question of hazard to human pregnancy is complicated by still other considerations. Although herbicides are of economic benefit to man, their use is not without possible hazard to the environment and to other aspects of human welfare. In various connections questions have been raised about: 1) damage to non-target plants caused by spray drift or by movement in water, 2) damage to subsequently planted sensitive crops owing to herbicide persistence in the soil, and 3) acute or chronic toxicity to man or other animals aside from that related to pregnancy. In addition, there is some concern that traces of the chemical or its contaminants in food may cause unsuspected effects in man or that minute amounts in the environment may adversely affect untested species in the ecosystem.

It is scientifically impossible to prove that a chemical is without hazard. Pesticide regulations now require that new agents be tested for acute and chronic toxicity, mutagenicity and carcinogenicity. These tests may involve the use of two or more species of animals taken through several generations and the examination of thousands of individuals. Since it is necessary to extrapolate from effects in test animals to man and since species are known to differ in sensitivity to chemicals, the permissible residue levels in food must always be many-fold below the minimal effect level for the species tested. Concern that some unexpected detrimental occurrence may outweigh the benefit of a pesticide has doubtless been heightened by the finding that DDT residues in the environment have adversely affected reproduction in certain predatory avian species that are at the top of their food chains and, as a consequence, ingest large accumulations of this persistent compound.

With these considerations in mind the Advisory Committee has examined all available information relating to factors that may influence human exposure to 2,4,5-T and the toxic reactions, nonteratogenic as well as teratogenic, such exposure of man and other animal species may entail.

I. FACTORS INFLUENCING EXPOSURE TO MAN.

Human exposure to an environmental chemical such as 2,4,5-T depends on 1) pattern of usage, i.e., how widely and frequently applied and in what amounts, and 2) its fate in the environment, i.e., does it accumulate or is it degraded as fast as applied.

A. Patterns of use.

The chloro-phenoxy herbicides 2,4-D and 2,4,5-T have been widely used to control broad-leaved weeds for over 20 years. Because 2,4,5-T is more expensive than 2,4-D (2,4-dichlorophenoxyacetic acid) it has been primarily used to control woody plants and a few herbaceous species against which it is more effective than 2,4-D, and because of the cost difference, commercial formulations containing 2,4,5-T are usually mixtures of the two herbicides. In 1964 the uses of 2,4,5-T were: rights-of-way - 49%, non-farm forests - 10%, hay, pasture, and rangelands - 7%, all other farm uses - 12%, lawns and turf - 7%, federal agencies - 6%, and other miscellaneous uses - 9%.[1/] The total domestic use at that time was about 9 million pounds and incomplete information indicates that this value may also be approximately correct for 1969.[2/]

Most of the 2,4,5-T used is applied as a spray to foliage. Lesser amounts are sprayed on the trunks and branches of dormant trees, injected into the bases of trees, poured or sprayed into frills around the trunks of trees, or sprayed or painted on newly cut stumps of trees. Amine salts of 2,4,5-T dissolved in water are most often used when the herbicide is applied to foliage and esters dissolved in oil are most

often used when it is applied to bark. The spray concentrations usually vary between 0.1 and 2.5% and the rates of application are usually between 0.5 and 8 lb per acre, depending on the size and sensitivity of the plants being treated. Higher rates and concentrations have been used in Vietnam for military purposes.

On domestic rice, 0.50 to 1.25 lb per acre of 2,4,5-T is used in 5 to 7 gal of water[3/], applied from the air when the rice is 7 to 9 weeks old, has emerged from the water, and is standing erect. Directions for the use of 2,4,5-T warn against allowing it to drift onto susceptible plant species and require that the herbicide not be allowed to contaminate water used for irrigation or domestic purposes.

B. Fate in soil, air, water and plants.

When 2,4,5-T is applied as a spray, the great bulk of the herbicide and any contaminant it may contain, e.g., 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD), are deposited on the foliage of the plants, on the ground in the immediate vicinity or, in the case of rice, on the impounded water. Much smaller amounts may be inserted into the air or settle on streams of water and by either means be carried many miles from the site of application. After 2,4,5-T and TCDD are applied, however, each moves through the biosphere and accumulates or degrades according to its own chemical and physical properties. The fate of 2,4,5-T has been more extensively studied than that of TCDD.

Fate of 2,4,5-T. Once the herbicide reaches the soil it is immediately subjected to physical and chemical actions that continually reduce the amount remaining at the site of application. These actions include degradation by soil microorganisms, leaching and surface

movement in water, volatilization, movement by wind, and photochemical decomposition. The persistence of 2,4,5-T is influenced by its rate of application and by various climatic and edaphic factors, and occurs most rapidly under conditions that are optimal for the growth of soil microorganisms.[4] At least two bacterial isolates, Mycoplano sp. and Achromobacter sp.[5][6][7][8] and one actinomycete, Streptomyces viridochromogenes[9] from soil are known to metabolize 2,4,5-T. Brevibacterium sp. has been shown to cometabolize 2,4,5-T to a product tentatively identified as 3,5-dichlorocatechol[10]. Norris[11] found that 2,4,5-T was decarboxylated in the litter of the forest floor and had a half-life of approximately 40 days. Loos[12] has thoroughly reviewed degradation of phenoxyalkanoic acids, including 2,4,5-T. Loss of all phytotoxicity of 2,4,5-T applied to the soil was reported to occur 3 to 6 months after application.[13] No chemically detectable amounts of 2,4,5-T were found in the soil 1 year after an application of 2 lb per acre and only very small amounts were found 3 to 7 months after application.[14] Although the rate of disappearance varies, there have been no reports of carry-over of 2,4,5-T from one year to the next, indicating that no build-up in the soil would result from recommended rates of treatment repeated annually.

When phenoxy herbicides were first introduced, highly volatile esters were available and farmers were inexperienced in their use, there were several instances in which the drift through air produced severe injury to sensitive crops, usually in adjoining fields or more rarely at some distance from the point of application. Owing to accumulated experience and the institution of regulations regarding the conditions under which applications can be made, as well as the removal of the volatile esters from the market,

injury to crops is now unusual. The elimination of drift sufficient to injure most crop plants, however, does not eliminate the possibility of drift that can be detected chemically.

Some of the 2,4,5-T applied as spray can be transported in the atmosphere as droplets of spray, as the gaseous phase of 2,4,5-T, or adsorbed on dust or other particulate matter in the air. In a survey in the State of Washington, 2,4,5-T was detected 9 days out of 99 at Pullman, in average concentration in positive samples of 0.045 $\mu g/m^3$. At Kennewick it was found 14 days out of 102 at average concentration in positive samples of 0.012 $\mu g/m^3$ [14]. In Cincinnati, Ohio, 0.04 ppm was found adsorbed on dust in a trace of rain[15] presumably from applications in Texas. Photochemical degradation would be expected to occur in the air, particularly at high altitudes and in dry climates where ultraviolet radiation is highest. Kearney et al.[16] report that exposure of 5 and 10 ppm water solutions of 2,4,5-T to ultraviolet light from a 450 watt Hanovia lamp greatly reduced the 2,4,5-T present within 5 minutes. It is not possible to extrapolate accurately from these data to the rate of decomposition in sunlight, but it is obvious that photochemical degradation could play a significant role. Probably most of the 2,4,5-T that gets into the air very soon either settles out or is washed out by rain and thereby is returned to soil and water. There is no evidence to suggest that 2,4,5-T remains in the air for more than a few weeks after insertion.

Measurable quantities of 2,4,5-T could enter water in several ways, e.g., by inadvertent direct spraying, from surface leaching of treated soils and plants, or in rain that falls through air containing 2,4,5-T; but undoubtedly most of it is washed from treated plants and soil.

Apparently the amounts are usually quite small since only 28 of 322 water samples taken in western states, where 2,4,5-T is widely used for brush control, were shown to contain 2,4,5-T[17] in concentrations ranging from 0.01 to 0.07 ppb. In closely controlled watershed studies in Waynesville, North Carolina, no 2,4,5-T was found in any sample of run-off from an area one-fourth of which was treated with 2 lb of 2,4,5-T per acre in 1968 or 1969[18]. When one-fourth the area was treated with 4 lb per acre some herbicide was found in the water after the first and second rain storms, but the highest concentration found was 0.048 ppm in run-off water during a storm that occurred 8 days after application of the herbicide. No 2,4,5-T was found in the last sample collected that day or in those collected on subsequent days.

Few data are available on the rate of disappearance of 2,4,5-T from water. It would be expected to be adsorbed on clay particles or adsorbed by aquatic species within a few days. The concentration of picloram, a considerably more persistent herbicide than 2,4,5-T in most situations, decreased from 0.965 ppm to 0.129 ppm in 3 weeks in a test in which it was applied at the rate of 4 lb per acre to a pond[19]. All available data suggest that the amount of 2,4,5-T entering water is quite low and that it does not remain in the water very long.

Absorption, translocation, and metabolism of 2,4-D and 2,4,5-T by plants have been extensively investigated. Most investigations have dealt with 2,4-D, but numerous studies have involved both and it is apparent that their behavior in plants is similar. Ready absorption of 2,4,5-T by leaves, stems, and roots of plants is known to occur.[20][21][22][23][24][25] Once absorbed 2,4,5-T may either move upward in the xylem or bidirectionally in

the phloem. Some 2,4,5-T is absorbed by the leaves, transported down the stem and into the roots, and excreted by the roots into soil solution[22/].

Decarboxylation of 2,4,5-T has been demonstrated in a number of plant species[12/]. The varied sensitivity of different species to 2,4,5-T may be attributable in part to different rates of metabolism. Slife et al.[25/] found only traces of unidentified metabolites 8 days after applying ^{14}C-carboxyl-labeled 2,4,5-T to wild or cultivated cucumber plants, both species susceptible to 2,4,5-T. Basler and associates[26/27/] applied ^{14}C-carboxyl-labeled 2,4,5-T to excised blackjack oak leaves. They found no decarboxylation but did find that an average of 59% of the 2,4,5-T was broken down into three major unidentified metabolites in 24 hours. Morton[28/] reported that 80% of 2,4,5-T applied to mesquite was metabolized in 24 hours. Fitzgerald et al.[29/] identified 2,4,5-trichlorophenol as a common metabolic product of 2,4,5-T in sweetgum and southern red oak but found that no 2,4,5-trichloroanisole was formed. Morton et al.[23/] have studied the metabolism of various formulations of 2,4,5-T by beardgrass, little bluestem, and sideoats gramma and observed only moderate effect of formulation and species on the rate of metabolism. Half-life values in green tissues ranged from 1.6 to 2.9 weeks. In one experiment using radioactive 2,4,5-T ester and silver beardgrass, little bluestem, and dallis grass, alcohol extracts of green tissue taken 1 week after application contained no 2,4,5-T ester, 50% 2,4,5-T acid, and 50% unknown radioactive metabolite.

Authorization to use 2,4,5-T on food crops depends on demonstrating that no residue exists in the edible product at harvest. The following studies illustrate the amounts of 2,4,5-T that may persist in food crops at various intervals after treatment. When 2,4,5-T was applied to apples as a

spray concentration of 40 ppm, residue in the fruit had fallen to 0.004 ppm in 22 days.[30] The application of 2,4,5-T to blueberries at 1 lb per acre resulted in a concentration in the fruit of 0.05 to 0.33 ppm 44 days after application although none was found 733 days after application.[31] No detectable 2,4,5-T (sensitivity = 0.01 ppm) was found in rough rice 50 days after applying 2.25 lb per acre of 2,4,5-T.[32] The rice straw contained 0.18 to 1.04 ppm 2,4,5-T 50 days after but none 84 days after application.

Further evidence that very little 2,4,5-T gets into food is seen in results of assays of raw agricultural products and in the Market Basket Survey samples. From about 10,000 food and feed samples examined from 1964 through 1969 only 25 contained trace amounts of 2,4,5-T (less than 0.1 ppm) and only two contained measurable amounts, 0.19 ppm in a sample of milk in 1965 and 0.29 ppm in a sample of sugar beets in 1966.[33] Furthermore, of the 134 total diet samples involving 1600 food composites (Market Basket Survey) analyzed from 1964 through April 1969, only 3 contained 2,4,5-T. Two were dairy products containing 8 to 13% fat with 0.008 and 0.19 ppm in the fat. A single meat, fish and poultry composite from Boston consisting of 17 to 23% of fat was found to contain 0.003 ppm 2,4,5-T on a fat basis.[33/34/35]

It is concluded from the foregoing that: 1) The herbicide 2,4,5-T does not accumulate in any compartment of the biosphere. 2) The risk of human exposure to 2,4,5-T in food, air or water is negligible.

Fate of TCDD. Under present conditions of manufacture this contaminant is usually present in 2,4,5-T at less than 1 ppm, thus insuring that very little TCDD is inadvertently applied with 2,4,5-T. Like 2,4,5-T any contaminating TCDD would be deposited on the leaves of treated vegetation

or on soil and water in the vicinity, although as indicated for 2,4,5-T, smaller amounts could enter air or water and be carried some distance from the site of application. Water transport, however, is sharply limited by the fact that the solubility of TCDD in water is only 0.2 ppb.[36/] As a consequence it would tend to remain on the surface of plants and soil at the site of application.

Photochemical decomposition of TCDD has been studied at Dow Chemical Co.[37/] and the United States Department of Agriculture.[38/] Exposure of 1.02 mg of TCDD in 100 ml of water-saturated chloroform to ultraviolet light at 35°C caused 50 to 100% degradation in 2.5 hours. The Department of Agriculture, using a sunlamp with a peak emission at 310 nm, irradiated TCDD dissolved in methanol and found it to have a half-life of 3.5 hours. Rapid decomposition was also reported in natural sunlight when approximately 5 ml of a 24-ppm methanol solution of TCDD was sealed in glass tubes and exposed to 7,000 to 9,000 footcandles of sunlight, with a half-life of approximately 5 hours, virtual disappearance in 48 hours, and none detectable after 72 hours. Similar rates of decomposition, however, were not observed when the TCDD was placed on the surface of dry soil where irradiation for 96 hours with a sunlamp (maximum energy at 310 nm) did not cause any significant loss by either photodecomposition or volatilization. The same was true for wet soil irradiated for 6 hours.

Interactions of TCDD with soil have also been studied by the United States Department of Agriculture.[39/] When TCDD was placed on the surface of five very different soils and subjected to leaching with water, the TCDD did not move into any of the soils, probably because of its very low water solubility. Similarly, in leaching experiments using soil thin-

layer chromatographic technique, no TCDD moved from the spot of origin in either a Hagerstown silty clay loam or a Norfolk sandy loam.[38/] It would thus appear that most of the chemical falling on the soil surface would remain there. The fate of TCDD mechanically incorporated into soil has been investigated by the Department of Agriculture.[39/] Radiolabeled TCDD was mixed into soil at the rates of 1, 10, and 100 ppm and soil extracts radio-assayed 20, 40, 80, and 160 days after application. The amount of radioactive material (probably TCDD) in the soil decreased 15 to 20% in 160 days, indicating that this compound was very slowly degraded in the soil and could persist for more than a year.

The possibility that TCDD incorporated in the soil might be absorbed by plants has been studied.[39/] Soybean and oat plants were grown on Lakeland sand containing 0.06 ppm radiolabeled TCDD, which is 40,000 times the amount that would appear in soils treated with 2 lb per acre of 2,4,5-T containing 1 ppm TCDD. Less than 0.2% of the available TCDD was absorbed by either type of plant, with radioactivity reaching a peak at 10 days at which time it measured about 0.12 ppm in oats and 0.05 ppm in soybeans on a dry weight basis, then it declined to an insignificant level at 40 days.

The most likely source of plant contamination by the TCDD present as a contaminant in commercial 2,4,5-T is by way of foliar application. When radioactive TCDD was applied to the surface of leaves[39/] no material was translocated from the site of application on the plant; but about 40 percent of the applied TCDD could be leached from the surface of the leaves by water, probably because the TCDD was added with a surfactant. This suggests that surface contamination could be the source of a very small TCDD residue in leafy food plants, but 2,4,5-T is not used on such

plants. Even if 2,4,5-T containing 1 ppm TCDD were applied to such plants at the rate of 2 lb per acre, the resultant TCDD contamination would be at the extremely low level of 0.224 μg per m^2 on the exposed surface.

It is concluded from the foregoing that: 1) There is no indication that TCDD accumulates in air, water or plants, although it might accumulate in soils after heavy application of a highly contaminated sample of 2,4,5-T. 2) Direct application of 2,4,5-T containing TCDD could result in minute quantities of the latter remaining on the surface of foliage. 3) Less than 0.2% of TCDD in soil is known to be absorbed into plants.

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C. Fate in Animals

Fate of 2,4,5-T. Information on absorption, distribution, and metabolism of 2,4,5-T is not extensive. The most thorough studies are those reported by Erne [1/2/] who demonstrated that the triethanolamine and alkaline salts of 2,4,5-T and 2,4-D were readily absorbed, distributed and eliminated from the body. Rats and pigs given single doses of 100 mg/kg of the amine salt showed plasma half-life values of 3 and 10 hours respectively. Residues in kidney, liver, lungs and spleen sometimes exceeded plasma levels, but there was little indication of penetration into brain or adipose tissues. The compounds were excreted mainly via the kidney. With repeated administration plasma levels decreased and excretion rates increased. Up to 20% of the material in blood was in erythrocytes. As with single doses, little was found in adipose tissue or in the central nervous system. Placental transfer was found to be rapid in swine. Tissue half-times ranged from 5 to 30 hours and were lowest in rats. There was no apparent retention of either 2,4-D or of 2,4,5-T after repeated administration.

According to St. John et al., [3/] when a cow was given 450 mg of 2,4,5-T acid divided among four daily doses, all of the administered material was excreted in the urine as the salt within 6 days. Zielinski and Fishbein [4/] found that a dose of 100 mg/kg of 2,4,5-T in mice was lost from the body more slowly than were several other herbicides, with disappearance rates ranging between 1 and 4% of the original dose per hour.

The rate of excretion of 2,4,5-T in man is unknown, but it seems to be slower than that of 2,4-D. A man who committed suicide with a mixture of the two compounds had substantial concentrations of 2,4,5-T in all organs analyzed but no 2,4-D in any organ [5/].

Using massive doses it is possible experimentally to exceed the ability of domestic animals to eliminate 2,4,5-T and thereby to produce measurable residues in their tissues. Four or more 250 mg doses of 2,4,5-T given to sheep produced levels of 33 to 113 ppm in fat and 40 to 100 ppm in muscle. The residues were 99% or more in the acid form regardless of whether the acid form or an ester was fed [6/] The chemical form of the agent, however, may influence its deposition. Oral administration of the propylene glycol or butyl ether esters of 2,4,5-T to yearling cattle for 32 weeks at rates of 0.15 and 0.75 mg/kg/day produced no residues greater than those occasionally found in untreated controls. Subsequently Clark et al., [7/] fed 2,4,5-T, 2,4-D and Silvex (2,4,5-trichlorophenoxy propionic acid) to sheep and cattle at several dietary concentrations for 28 days. Sheep receiving 2000 ppm 2,4,5-T in the diet were found to have muscle tissue residues of 1.0 ppm when treatment was terminated, and no detectable residue 7 days later. Newton and Norris [8/] analyzed tissues from deer that had ranged over reforested land treated with 2,4,5-T and found essentially no residues.

Several factors limit the intake of 2,4,5-T by domestic animals and man following recommended use of the compound, namely, low rate of application and breakdown by plants, animals, photochemical degradation and soil microorganisms. Owing to both the limited nature of prescribed use and the decomposition that occurs in the environment, 2,4,5-T almost never reaches a detectable level in human drinking water or food (see Section I B, pp. 11 and 14). Examination of approximately 11,600 samples of food offered for retail sale in the United States revealed only five

with measurable residues, the highest concentration being 0.29 ppm. The highest level in potable water was 0.00007 ppm [9/10/11].

Fate of TCDD. Piper and Rose [12] have reported a preliminary study of the tissue distribution and disposition of ^{14}C-labeled TCDD administered as a single oral dose of 0.05 mg/kg to male rats. The biological half-time for this dose was approximately 20 days, and fecal excretion accounted for the greater part of the TCDD removal. Three days after administration 3.1% of the total dose per gram was recovered from liver and 3.0% of the total dose per gram was contained in fat. The residual radioactivity in these tissues was not identified and therefore cannot be assumed to be TCDD. The fact that 8.3% of the dose was recovered from $^{14}CO_2$ in expired air indicated that some of the TCDD was completely metabolized.

The Dow Chemical Co. has recently provided comparative solubility data on TCDD and p,p'DDT at 24°C, as follows:

	PPM of Solvent	
	TCDD	p,p'DDT
corn oil	28	86,000
lard oil	44	86,000
water	0.0002	0.001

Although suggesting a petitioning toward fat, these data clearly indicate that, unlike DDT, TCDD is so insoluble in fat that it would not be expected to accumulate in body fat depots in appreciable amounts.

It is concluded from the foregoing that: 1) 2,4,5-T is rapidly excreted in all animals studied using doses in the range of those likely to be encountered in the environment; 2) 2,4,5-T is not known to be accumulated in any animal tissues or product used for human food;

3) 2,4,5-T has been detected in animal tissues or products used for human food very infrequently and then only in minute quantities; 4) limited data indicate that TCDD is also eliminated, at least some by metabolic breakdown, with a half-life of 20 days; and 5) the solubility of TCDD in fat is limited which would preclude appreciable accumulation in body fat.

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II. TOXICITY OF 2,4,5-T AND TCDD IN ANIMALS AND MAN

Currently available commercial preparations of 2,4,5-T can be characterized as having at least 95% 2,4,5-T with less than 0.5 ppm TCDD and no other toxicologically significant compound. Many earlier studies on the adverse effects of 2,4,5-T employed preparations containing considerably greater concentrations of TCDD than this, and others used 2,4,5-T samples of unspecified purity. Toxicological studies utilizing 2,4,5-T preparations which were not known to conform to the standards suggested above, nevertheless, have some value because any error attributable to larger amounts of TCDD would have been toward the conservative side, that is, would have suggested greater toxicity than if a purer 2,4,5-T had been used.

A. Nonteratogenic Toxicity.

Of 2,4,5-T. Among the earlier reports of 2,4,5-T toxicity that did not fully identify the composition of the product under investigation were the studies of Drill and Hiratzka[1/] in which oral LD_{50} for dogs was estimated to be in excess of 100 mg/kg, and of Rowe and Hymas[2/] in which the oral LD_{50} to various rodents was found to be greater than 350 mg/kg. Drill and Hiratzka found no adverse effects in dogs which were fed 2,4,5-T five times a week for 90 days at dosage levels of 2.5 and 10 mg/kg. Four dogs were treated at a level of 20 mg/kg 2,4,5-T and died at 11, 49, 59 and 75 days after the first dose. Rowe and Hymas reviewed the toxicologic information available on 2,4,5-T at that time, and concluded that the acute lethal oral toxicity, in terms

of LD_{50} and 19/20 confidence limits, of 2,4,5-T was for male rats 500 (391-640) mg/kg; for male mice, 389 (245-619); guinea pigs, male and female, 381 (307-472); chicks, male and female, 310 (211-456). The latter authors also used various commercial formulations of the butyl, isopropyl and amyl esters of 2,4,5-T in single oral-dose animal-feeding experiments in rats, chickens and guinea pigs and reported LD_{50} levels with 19/20 confidence limits which were all greater than those listed above. They concluded that oral administration of 2,4,5-T could be tolerated without adverse effects in doses only slightly smaller than those which caused toxic effects and stated that this fact demonstrates that 2,4,5-T has a low degree of chronicity. Palmer and Radeleff[3/] found that the propyl glycol butyl ether ester of 2,4,5-T was lethal to one sheep after 369 daily oral doses of 100 mg/kg and to another sheep after seven doses of 250 mg/kg. A single cow also succumbed to the latter dose. The triethylamine salt of 2,4,5-T caused no observed effect (sheep) after 481 doses of 100 mg/kg/day. The propionic acid butyl ether ester of 2,4,5-T was lethal to a sheep after 11 daily doses of 100 mg/kg orally and lethal to a cow after 29 such doses. Five daily 250 mg/kg oral doses also killed a cow. Fifty mg/kg/day orally had no effect after 73 days.

In 1969 an investigation of the carcinogenicity in mice of 120 pesticides and herbicides[4/]; 2,4,5-T was among the compounds tested which did not cause significant increase in tumors after oral administration. The purity or dioxin content of the sample was not described. This represents the only report of long-term treatment with 2,4,5-T other than the few farm animals mentioned above. The dose was the maximum

tolerated dose (zero mortality) determined with single doses, 6 daily doses, and finally 19 daily doses. The dosage was given by stomach tube at 21.5 mg/kg from the end of the first through the fourth weeks and thereafter it was mixed in the diet at 60 ppm of food and continued until 18 months of age. It is presumed that all animals survived the 18 month test period although this was not stated in the publication.

Johnson[5/] has reported acute oral, single dose toxicity studies on commercial 2,4,5-T in which the LD_{50} for 2,4,5-T was 500 mg/kg in the rat and 380 mg/kg in the guinea pig. Ninety-day feeding studies with 2,4,5-T containing 0.5 ppm TCDD were recently reported by McCollister and Kociba.[6/] The acid form was administered to groups of 10 male and 10 female rats at 100, 30, 103 and 0 mg/kg/day. No significant adverse effects were observed in the groups receiving doses at or below 30 mg/kg/day, but those receiving 100 mg/kg/day showed a depression of body weight gain, a decrease in food intake and elevated alkaline phosphatase levels. The males showed slightly increased serum glutamic-pyruvic transaminase levels and slight decreases in red blood cell counts and hemoglobin levels. Histological evidence of toxicity were minor and inconsistant. In an earlier experiment at Dow Chemical Co.[7/], the mono-, di-, and tripropylene glycol butyl ether esters of 2,4,5-T were administered orally to rats over a similar 90-day period at doses as high as 186 mg of 2,4,5-T acid equivalent per kg per day. At the highest dose and at 62 mg/kg/day of acid equivalent various evidences of toxicity developed, but no adverse manifestations attributable to the agent were detected at dosages of 18.6 or 6.2 mg/kg/day.

The Dow Chemical Co.[8/] has prepared an extensive health inventory of 126 manufacturing personnel in an effort to identify adverse effects

of inhaled 2,4,5-T. The inhalation rate of the agent was estimated to be 1.6 to 8.1 mg/day per worker, depending on the work assignment, for periods of up to three years and at total career exposures in excess of 10,000 mg. The survey indicates that no illness was associated with 2,4,5-T intake. Specifically there was no increase in skin ailments or of alkaline phosphatase or SGPT levels as compared with controls having no exposure to 2,4,5-T.

The result was entirely different in a plant where the 2,4,5-T produced contained a high proportion of dioxin. The latter plant was studied by Bleiberg[9/] in 1964 and again six years later by Poland et al.[10/] who also reviewed earlier studies in factories in other countries where TCDD had been a problem. Poland and associates reported on 73 employees whose health was found to be improved compared to that of workers in the plant six years earlier. Eighteen percent of the men had suffered moderate to severe chloracne, the intensity of which correlated significantly with the presence of residual hyperpigmentation, hirsutism, and eye irritation and with a high score on a test indicating a manic reaction. The chloracne did not correlate with job location or duration of employment at the plant or with coproporphrin excretion. One of the men had uroporphyrinuria but, unlike the situation six years earlier, no porphyria could be found. Systemic illness such as may be produced by TCDD was markedly less than that reported in previous studies of 2,4,5-T plants and probably no greater than expected in unexposed men of the same age.

Dogs and rats tolerate oral intake of 2,4,5-T at a rate of 10 mg/kg/day or higher without detectable clinical, biochemical, or pathological change. The tolerance limit of people is not known but

no injury was detected in workers with the highest recorded, prolonged exposure in a factory making low-dioxin 2,4,5-T, i.e., 8.1 mg/man/day or about 0.11 mg/kg/day[8]. In view of the small and highly infrequent occurrence of residues of 2,4,5-T in human food (see Section I B), it is clear that exposure from this source is too small to measure accurately. Thus, although it is impossible to estimate how much greater the 2,4,5-T exposure of workers is than the exposure of the general population, it is clearly much greater than the corresponding one for DDT[11]. In fact, exposure to 2,4,5-T is trivial even for persons who daily eat unpolished rice.

The very small number of cases in which human ingestion of 2,4,5-T led to clinical illness offer no information on the minimal dosage of the compound that is toxic to man. In animals, however, the toxicity of 2,4,5-T is similar to that of 2,4-D, consequently some information on 2,4-D is of interest. When 2,4-D was investigated as a possible treatment for disseminated coccidiodomycosis, the patient had no side-effects from 18 intravenous doses during 33 days; each of the last 12 doses in this series was 800 mg (about 15 mg/kg) or more, the last being 2000 mg (about 37 mg/kg). A 19th and final dose of 3600 mg (67 mg/kg) produced mild symptoms[12]. Suicidal ingestion of a quantity of 2,4-D as a single dose known to be greater than 6500 mg (in excess of 90 mg/kg) was fatal[13].

Butler[14][15] has reviewed Fish and Wildlife Service studies of pesticide and herbicide effects on marine organisms. Several 2,4,5-T derivatives were examined (TCDD content was not known). A 96-hour exposure of oysters to the polyglycol butyl ether esters of 2,4,5-T at a concentration of 0.14 ppm in the water caused a 50% decrease in shell

growth rate, with recovery in one week. The 24-hour LD_{50} of this ester to juvenile estuarine fish was 0.32 ppm. A concentration of 2,4,5-T acid at 2 ppm caused no decrease in growth after 96 hours. A level of 50 ppm 2,4,5-T was not lethal to juvenile mullet and killifish in 48 hours, and 1 ppm was without effect on shrimp in 48 hours. It is thus apparent that aquatic species tolerate higher concentrations of 2,4,5-T than have been reported in water samples taken from heavily sprayed areas (see Section I B, pp. 11-12).

It is concluded from the foregoing that: 1) most species tested can survive a single oral dose in excess of 100 mg/kg and several, excepting the dog, can survive daily treatment for a number of days at this level or higher; 2) dogs die after 11 to 75 doses at the rate of 20 mg/kg/day and rats show toxic signs at repeated daily doses of 100 mg/kg; both species tolerate 10 mg/kg/day without detectable effect; 3) no proven instance of toxicity associated with 2,4,5-T intake in man has been found in industrial or agricultural workers known to have had repeated, relatively high levels of exposure to 2,4,5-T of low dioxin content; and 4) the safety factor for the general population is estimated to be several orders of magnitude greater than that of 2,4,5-T factory workers.

Of TCDD. TCDD has been recognized as a contaminant of commercial preparations of 2,4,5-T for several years; however, there has been no extensive study of its toxicity. According to Johnson[13] the acute LD_{50} for TCDD is 0.022-0.045 mg/kg in the rat and 0.0006 mg/kg in the guinea pig. Because of the high potency of this compound in the guinea pig, these experiments were repeated and confirmed by

Dow Chemical Co. Some information on the toxicity of TCDD is available from a study of TCDD teratogenic effect in the rat (see Section II B, p. 44). No evidence of clinical effect on the dams was found at doses of 0.0005 mg/kg/day, although embryotoxicity appeared in litters of females given 0.000125 mg/kg/day. Some vaginal hemorrhage was caused by 0.002 mg/kg/day and 0.008 mg/kg/day caused pallor and debilitation.

As far as occupational exposure is concerned, it is clear that any danger of 2,4,5-T formulations resides in their TCDD content. The primary manifestation of industrial TCDD intoxication is chloracne, an easily detected, in fact highly disfiguring, dermatitis. It is significant that this condition has not been a problem in factories producing 2,4,5-T with a low content of TCDD, nor among persons who apply the herbicide as a part of their regular occupation. It is therefore highly unlikely that exposure to traces of TCDD will have any effect on persons who use 2,4,5-T formulations occasionally or who merely encounter possible traces of it in the environment.

Data are too limited for a firm conclusion but there is no evidence to suggest that TCDD as a contaminant in 2,4,5-T is likely to be encountered by animal or man in sufficient dosage to cause toxic reactions.

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B. The Teratogenic Potential of 2,4,5-T

1. Scope of embryotoxicity. Teratology is the science dealing with the causes, mechanisms, and manifestations of deviant structural or functional development. Such deviation can be the result of mutation in which case the defect may be transmitted by heredity, or it may be induced by unfavorable environmental conditons during the developmental period: usually during the formative stages of the embryo, less often during functional maturation of the fetus, and possibly even during the final stages of development postnatally.

Many types of adverse factors in the environment have been shown to initiate abnormal development when applied during pregnancy in laboratory animals, including: certain dietary deficiencies (mostly of vitamins); many classes of chemicals, including some drugs; various physical factors such as ionizing radiation, drastic temperature changes, and alterations in atmospheric gases; a few viral infections; some maternal endocrine and metabolic imbalances; and undoubtedly some combinations of these.

Relatively few of these experimentally demonstrated teratogenic agents have been shown to be effective in man. High doses of ionizing radiation such as are used in therapy for cancer or emanate from nuclear explosions are well known to be teratogenic when applied during early human pregnancy. Two infectious agents, rubella and cytomegalic viruses, have been clearly implicated. Three types of drugs - thalidomide, folic acid antagonists, and androgenic hormones - have been established as causes of malformations in man and a few others are suspected but are not at this time proven to be teratogenic. Maternal metabolic diseases

such as endemic cretinism, diabetes, phenylketonuria, and adrenal hyperplasia account for a small percentage of human developmental disease. One environmental pollutant, methylmercury, proved to be teratogenic for man when it reached high concentrations in certain water in Japan from which fish were eaten as a large part of the diet.

Adverse effects on development are difficult to evaluate because they vary greatly in degree and type. Collectively these effects can be designated as embryotoxic because they most often have their inception in the embryo and include such manifestations of toxicity as lethality, teratogenicity, prenatal growth retardation, and postnatal functional deficiencies. Few attempts are made to evaluate functional deficiency except as it may be reflected in postnatal survival data. On the other hand embryolethality, teratogenicity, and growth retardation can under laboratory conditions be readily detected and quantitated. Difficulty is often encountered, however, when all three toxic manifestations are simultaneously evaluated, since the phenomena involved are rarely affected to the same degree by a given embryotoxic agent. Although most chemical substances probably could be shown to be teratogenic under suitable experimental conditions and all could be shown to have some toxic effects when dosage is sufficiently high, some would be more strongly teratogenic, others would be predominantly embryolethal, whereas still others would tend mainly to cause intrauterine growth retardation. While these toxic manifestations vary directly with dosage, they may not show parallel dose-response effects. Any one of the three may begin to appear at a somewhat lower dose than either of the others. Lethality is probably the most variable from one agent to another, sometimes appearing at low doses and increasing slowly as dosage is increased, sometimes

appearing abruptly at doses already causing considerable teratogenicity and growth retardation. Teratogenicity is probably the most predictable of the three in that it usually has an easily demonstrable no-effect range of dosage and a steep dose-response curve once teratogenicity begins.

These variations in embryotoxic manifestations are particularly troublesome when it is necessary to establish the highest no-detectable-effect or the lowest effect level of dosage. A level that has no detectable teratogenic effect may already be in the effect range for lethality or growth retardation. The solution to this dilemma requires either that one form of embryotoxicity be selected as the criterion of interest or that the one showing the lowest effect level arbitrarily be accepted in setting tolerance limits. Such complications have been encountered in attempting to evaluate 2,4,5-T results, particularly in those experiments in which data on all embryotoxic manifestations were not reported. In some experiments only results pertaining to teratogenicity were given and in these cases dose-response evaluation had to be limited accordingly. For others, the Advisory Committee has tried to extrapolate the data in such a way as to approximate a no-effect level, i.e., the largest dose at which no increased lethality, teratogenicity, or growth retardation occurred.

2. Data from laboratory animals. In 1964, the National Cancer Institute contracted with Bionetics Research Laboratories to perform screening studies for carcinogenicity and teratogenicity on a number of pesticides and industrial chemicals. The results, released in October 1969, indicated that of the 53 compounds examined, 2,4,5-T in particular showed embryotoxicity in two stocks of mice at a dose of 113 mg/kg/day

when given for several days during organogenesis. Cleft palate, cystic kidneys, intestinal hemorrhage and fetal mortality occurred in higher percentages of treated than of control animals although a clear dose-response relation was not evident at lower doses. The results have been reviewed elsewhere [1/2/] and published in summary form [3/] and therefore require no extensive discussion here. Certain inconsistencies in the data [2/4/] likewise need no comment because the sample 2,4,5-T used in the Bionetics study is known to have been contaminated with 27 $\pm$ 8 ppm of TCDD and the results can no longer be considered a valid indication of the teratogenicity of the herbicide. This contaminant itself has since been shown to have teratogenic and embryolethal properties, as will be discussed later. Despite the limitations of the original Bionetics study, it served two useful functions, in: 1) highlighting the possibility that herbicides may cause previously unknown adverse effects on nontarget organisms, including mammals, and 2) emphasizing the need for more thorough safety evaluation of such compounds before they are approved for widespread use.

The discovery that the contaminant TCDD was present in the herbicide used in the Bionetics study made it necessary to determine whether the reported teratogenicity was caused by 2,4,5-T or TCDD. Additional studies relating to this question have been completed at the Dow Chemical Company, the Food and Drug Administration, the National Institute for Dental Research, the National Institute of Environmental Health Sciences, the Department of Agriculture Animal Disease and Parasite Research Division, the Food and Drug Directorate of Canada, Bionetics Research Laboratories, and

the Children's Hospital Research Foundation of Cincinnati, on rats, mice, hamsters, rabbits, sheep and rhesus monkeys using samples of 2,4,5-T containing known concentrations of TCDD as well as relatively pure samples of TCDD.

These studies are summarized below, species by species and separately for 2,4,5-T and TCDD. Insofar as the original reports permits, data are summarized on maternal toxicity, e.g., death or failure to show normal weight gain during pregnancy; as well as on embryotoxicity and fetal toxicity, e.g., prenatal death teratogenesis, intrauterine growth retardation, and perinatal signs of other toxicity. It is recognized that fetal death, either individual or as whole litters may also reflect maternal toxicity, and therefore may be difficult to interpret.

2,4,5-T in rats. Sprague-Dawley rats at Dow Chemical Co. were fed 1, 3, 6, 12, or 24 mg/kg/day of 2,4,5-T containing 0.5 ppm of TCDD on days 6 through 15 of pregnancy [5/6/7]. No maternal death or reduced maternal weight gain during pregnancy was noted. There was also no increase in prenatal mortality, only slight impairment of fetal growth in a few cases at the 24 mg/kg dosage, and no malformations. The poor ossification of the 5th sternebra noted in some cases was probably a sign of mild transient retardation of skeletal development and of no known significance. Pregnant rats of the same stock were fed 50 or 100 mg/kg/day of "commercial production grade" 2,4,5-T containing 0.5 ppm TCDD on days 6 through 15 of gestation, or 100 mg/kg/day on days 6 through 10 [8/9]. The only effects observed after the lower dose were intestinal hemorrhage in one of 203 offspring and a slight increase in

frequency of delayed ossification of skull bones. The larger dose produced 83% maternal death and early death (resorption) of the entire litters in most of the surviving pregnant animals. Surviving offspring were reduced in size but had no anomalies except delayed ossification of skull bones, and this retardation was overcome within three weeks after birth.

Sprague-Dawley rats at the National Institute of Dental Research [10/11/] were given orally 60, 80, 100, or 120 mg/kg/day of 2,4,5-T containing 0.4 ppm TCDD over various periods of consecutive days during the middle third of gestation. Maternal toxicity data were not reported. No treatment greatly increased the intrauterine mortality rate and many had no effect. Prenatal growth retardation was not mentioned. Apparently the offspring were examined only for external malformations and those of the oral cavity. Very few with such defects were found (7 of 1500 from females treated at susceptible periods). A mixture of 2,4,5-T and 2,4-D produced one case of cleft palate. To rule out the possibility that the chemical might not be reaching the fetus, millipore filters soaked with 0.05, 0.1, 0.11, or 0.125 mg 2,4,5-T were applied to amniotic sacs on day 12, 13, 14, 15, or 16 of gestation. Of 68 fetuses surviving to the time of examination two had cleft palate, one had a tail defect, four had limb defects, and others were small or edematous. A second study of this type yeilded one possible limb defect and four possible tail defects in 68 survivors.

Rats of the FW-49 stock in Germany recently were given 25, 50, 100, or 150 mg/kg/day of 2,4,5-T (containing <0.02 ppm TCDD) on days 6 through 15 of gestation (cited by Tschirley [12/]). Macro-and Microscopic examination revealed no signs of teratogenicity even with the highest

dosages. There was an increase in the prenatal mortality beginning at the 50 mg/kg dose and a reduction in the mean fetal weight beginning at the 100 mg/kg dose. Complete details of the study were not given.

Charles River rats at the National Institute of Environmental Health Sciences [13/14/15/] received samples of 2,4,5-T (0.5 and 30 ppm TCDD) at the rate of 10 to 80 mg/kg/day orally or subcutaneously on days 6 through 15 of gestation, or 2,4,5-T (< 0.05 ppm TCDD) at the rate of 150 mg/kg/day subcutaneously on days 14 and 15 of gestation. The 80 mg/kg dose was stated to be the maternal LD_{40} dose but data were not presented. The 80 and 150 mg/kg doses caused a reduction in maternal weight gain and increased prenatal mortality, but fetal weight was unaffected. A low incidence of fetal kidney anomalies was noted, but could not be attributed with confidence to the treatment. In addition, pregnant females were fed 50 mg/kg of 2,4,5-T (< 0.05 ppm TCDD) and allowed to deliver. The offspring examined periodically for 3 weeks postnatally during which time mortality, weight gain, and general development did not differ from those of control animals.

Wistar rats at the Food and Drug Directorate of Canada [16/] were fed dosages of 25, 50, 100, and 150 mg/kg/day of 2,4,5-T acid or of 2,4,5-T butyl ester containing <0.5 ppm TCDD on days 6 through 15 of gestation. No apparent adverse effects on pregnant females were noted, but fetal weight was reduced. At the largest dosage there was an apparent increase in the frequency of "spontaneously occurring" skeletal anomalies. The largest dose of the acid form killed 3 of 8 pregnant females and reduced maternal weight gain but lower doses were without maternal toxicity. Intrauterine death was increased at 50 mg/kg and was pronounced at larger dosages, at which levels weight of surviving

fetuses was reduced. At the higher doses there was also increased skeletal variations some of which did not occur spontaneously in controls. Postnatal survival of young was not adversely affected by maternal dosage with 100 mg/kg. The 2,4,5-T butyl ester was without effect.

The foregoing rat experiments all involved repeated daily treatment of pregnant females with 2,4,5-T. The possibility exists, that owing to maternal homeostatic mechanisms, e.g., inducation or inhibition of metabolic enzymes, the most sensitive teratological test would be one involving a single treatment during early organogenesis. To test this possibility rat experiments were carried out at the Institute of Developmental Research, Children's Hospital Research Foundation of Cincinnati [17]. Using a sample of 2,4,5-T containing 0.5 ppm of TCDD, groups of pregnant Wistar rats were treated by gavage on day 9 of gestation with doses of 100, 200 or 400 mg/kg in 0.2% carboxymethyl-cellulose. Day 9 is generally regarded as the time at which the rat embryo is teratogenically most susceptible.

Dose mg/kg	Days of treatment	Whole litters resorbed	Litters continuing to day 20			
			Total implants	% dead or resorbed	mean wt.of survivors	% survivors malformed[3]
Control[1]	none	0/40	509	5.4	3.7gm	1.9
Control[2]	9	0/45	558	7.2	3.8	0.8
20	7-13	0/11	170	8.8	3.7	0.7
100	9	0/11	170	9.0	3.7	1.9
200	9	0/11	156	11.5	3.8	5.1
400	9	2/10	122	25.4	3.2	11.0

[1]Cumulative untreated control over past 4 years.
[2]Cumulative vehicle treated control (per gavage) over past 4 years.
[3]Types of malformations: anophthalmia, microphthalmia, curly or short tail, hydronephrosis, ectopic testes, agnathia.

The data in the accompanying table indicate that a single dose of 100 mg/kg at a highly sensitive time in rat embryogenesis did not cause an increase in abnormal development or a decrease in intrauterine growth but did cause a slight increase in intrauterine death. This effect was accentuated at higher doses and a moderate increase in malformations above control levels was also noted. Intrauterine growth was affected only at 400 mg/kg, a dose sufficient to cause severe embryotoxicity as evidenced by complete resorption of 2 of the 10 whole litters.

In summary, it appears that rat strains vary considerably in their susceptibility to the embryotoxic effects of 2,4,5-T. A low level of teratogenicity may appear in some strains when repeated dosage exceeds 100 mg/kg/day, or single dosage on day 9 is at 200 to 400 mg/kg of maternal weight. Some increase in intrauterine death and decrease in intrauterine growth, as well as maternal toxicity, was sometimes noted at lower daily dosage, e.g., 50 mg/kg.

TCDD in rats. Rats have been treated during pregnancy with TCDD in appreciable dosage in only two laboratories. At the Dow Chemical Co. [18] pregnant Sprague-Dawley rats received 0.00003, 0.000125, 0.0005, 0.002, or 0.008 mg/kg/day of dioxin (91% TCDD) orally on days 6 through 15 of gestation. Only one maternal death occurred and maternal weight gain was depressed only by the largest doses. Prenatal mortality was greatly increased at the 0.002 mg/kg dosage and all fetuses were killed at the 0.008 mg/kg level. Fetal weight was greatly reduced at 0.002 mg/kg and somewhat reduced at lower doses. Only two offspring had possible malformations of the tail and limbs. Edema and intestinal hemorrhage were observed in some offspring of females treated with 0.000125, 0.0005, or 0.002 mg/kg. In a second Dow study [9] pregnant rats of a stock of unstated origin received

by an unstated route 50 mg/kg/day of "pure" 2,4,5-T (probably containing 0.05 ppm TCDD) to which was added 0.00001, 0.00003, 0.00006, 0.000125, 0.0005, or 0.001 mg/kg/day of TCDD on days 6 through 15 of gestation. Cleft palate occurred in ten litters, mostly in those receiving the 2,4,5-T plus 0.0005 or 0.001 mg TCDD. The frequency of offspring with cleft palate, as well as procedural details and toxicity were not described.

Charles River rats at the National Institute of Environmental Health Sciences [14/] received TCDD subcutaneously 0.0005 mg/kg/day on days 6 through 10 of gestation, or 0.002 mg/kg/day on days 9 and 10 or 13 and 14 of gestation. No malformations or excessive fetal mortality were noted; but various possible kidney anomalies and several instances of intestinal hemorrhage occurred. Thus, TCDD given to pregnant rats caused embryolethality and occasional teratogenicity at doses below the maternal toxic level.

2,4,5-T in mice. Mice of CD1, C57BL/6J and DBA/2J strains received, subscutaneously, 50, 100, 113, 125, or 150 mg/kg/day of 2,4,5-T containing <1, 0.5, or <0.05 ppm TCDD on days 6 through 15 of pregnancy at the National Institute of Environmental Health Sciences [14/]. No maternal death occurred and maternal weight was depressed only in C57BL mice at the 100 mg/kg dosage. Fetal mortality was increased only in CD1 mice at the 150 mg/kg level. Fetal weight was reduced in all three lines of mice at dosages of 100 mg/kg and greater. Cleft palate occurred in low but consistent frequencies in all three lines of mice at doses of 100 mg/kg and more with all three samples of 2,4,5-T. Where sufficient data were available, a dose-response relation for fetal mortality and growth retardation was noted. Paradoxically the frequency of kidney

anomalies, types unspecified, was increased above the low level of background occurrence by the 2,4,5-T containing 0.05 ppm TCDD, but not by the 2,4,5-T containing 0.5 ppm TCDD. In general, these mice showed a low level of teratogenicity at 100 mg/kg/day during embryogenesis, and some embryolethality and decreased fetal weight at lesser doses. Moore [19] found no appreciable difference in teratogenic and embryolethal potential between 2,4,5-T as free acid and its butyl, isooctyl and butyl ether esters at approximately molar equivalent dosage in mice.

Mice of the NIH all-purpose albino stock were given subcutaneously 113 mg/kg/day of 2,4,5-T containing 0.4 ppm TCDD or 113 mg/kg/day of a mixture containing 50% 2,4,5-T and 40% 2,4-D ("Orange") usually on days 6 through 14 of gestation at the National Institute of Dental Research [10]. Cleft palate occurred in 9 of 141 offspring, but no data regarding maternal toxicity and other fetal effects were reported.

In a recent study made by the Bionetics Research Laboratories, commissioned by Hercules Incorporated, CD1 mice were injected subcutaneously on days 6 through 15 of gestation with 100 mg/kg/day of 2,4,5-T supplied by the Dow Chemical Co. and Hercules Inc. No maternal death seems to have occurred and maternal weight gain was unaffected. Intrauterine mortality was not increased but mean fetal weight was slightly reduced. The only malformation that occurred was cleft palate and its frequency was 11.1% (27/243) with the Dow sample and 1.3% (3/235) with the Hercules sample. Both products had <0.5 ppm TCDD.

TCDD in mice. TCDD given to mice at 0.001 or 0.003 mg/kg/day subcutaneously on days 6 through 15 of gestation did not affect fetal mortality, maternal weight gain, or fetal weight, but did produce low frequencies of cleft palate in the three mouse lines used in the

National Institute of Environmental Health Sciences study 13/14/. In one experiment 2,4,5-T (100 mg/kg) and TCDD (0.001 mg/kg) given together apparently produced no greater frequency of cleft palate than when each was given alone. TCDD greatly increased the background rate of kidney anomalies, especially in C57BL mice. Thus the limited data indicate that TCDD has some teratogenic potential in mice at doses even lower than those causing appreciable intrauterine death.

2,4,5-T in hamsters. Golden hamsters of a commercially obtained stock were treated orally on days 6 through 10 of gestation with 20-100 mg/kg/day of 2,4,5-T from seven sources, at the Food and Drug Administration 20/21/ Four of the samples contained 45, 2.9, 0.5 and 0.1 ppm TCDD, respectively, and three contained no detectable TCDD. Information on maternal toxicity was not given. Fetal mortality was greatly increased by the TCDD-containing 2,4,5-T samples and its frequency was usually directly related to both 2,4,5-T dosage and dioxin content; but it was also moderately high and dose-related after 2,4,5-T containing no detectable dioxin. The mean weight of surviving fetuses was unaffected or only mildly so for the different samples. A low to moderate incidence of gastrointestinal hemorrhage was observed, but this was probably not developmental in origin. Malformations were noted in offspring exposed to 2,4,5-T containing TCDD or not, but their frequency was usually higher after 2,4,5-T containing dioxin than after 2,4,5-T that did not. No malformations were produced by 2,4,5-T alone below the 100 mg/kg dose, whereas all dosages of dioxin-containing 2,4,5-T produced malformations. Very few malformations (cleft palate, 2 cases, and ectopic heart, 1 case) resulted from use of dioxin-containing 2,4,5-T, and only at 100 mg/kg.

The most frequent defects, poorly characterized as "bulging eyes" and "poor head fusion", occurred in low percentage (15.8% and 11.4%, respectively) after 2,4,5-T with or without TCDD. Some apparent discrepancies were present in the calculations of the malformation rates. In addition 150 mg/kg/day of a recrystalized and extracted 2,4,5-T was used [22] and produced a high fetal mortality rate but no malformations.

TCDD in hamsters. Hamsters were given dioxin (21% tri, 53% tetra CDD) orally at 0.00013, 0.002 or 0.0091 mg/kg/day on days 6 through 10 of gestation at the Food and Drug Administration [22]. Maternal toxicity was not mentioned. Mean fetal weight was reduced only at the two highest dosages. Eye anomalies and prenatal mortality were most frequent at the highest dose. Gastrointestinal hemorrhage was noted at the 0.0005 and 0.002 mg/kg doses.

2,4,5-T in rabbits. New Zealand white rabbits were treated orally with 10, 20, or 40 mg/kg/day of 2,4,5-T containing 1 ppm TCDD on days 6 through 18 of gestation at Dow Chemical Co. [6][7]. No deaths of pregnant females occurred, and maternal weight gain and fetal mortality and weight were unaffected. No congenital malformations were noted and developmental variations were not increased in frequency.

2,4,5-T in sheep. Sheep were fed 100 mg/kg/day of Dow production 2,4,5-T or of Dow production 2,4,5-T propyleneglycolbutylether ester on days 14 through 36 of pregnancy at the Department of Agriculture Animal Disease and Parasite Research Division [9]. Two of 19 ewes died on days 35 and 36 of pregnancy, but their fetuses were normal. The other 17 delivered normal offspring at term. No further details were provided.

2,4,5-T in rhesus monkeys. The Poisons and Pesticides Board of

Sweden has commissioned a study in pregnant rhesus monkeys [23/] at doses of 5, 10, 20 and 40 mg/kg given three times per week for 4 weeks between days 20 and 48 of gestation. The sample of 2,4,5-T contained 0.5 ppm of TCDD. Twelve fetuses removed by hysterotomy at 100 days of gestation from females treated with one of the three lower doses (4 pregnancies each dose), were developmentally normal and fell within the range of weight for untreated fetuses of this age. Two of 4 pregnant females treated with the highest dose yielded normal fetuses and the other 2 have not been hysterotomized at this writing but are still pregnant. One femal treated at this level aborted on day 61 of gestation and the conceptus was too macerated for examination. Abortion rate among untreated females in this colony is about 7% prior to day 100 of gestation.

Summarizing available data on exposure of pregnant laboratory animals, it is notable that rats were used most often and under the widest variety of conditions. Pregnant females of several stocks received orally administered low-dioxin-content 2,4,5-T in doses up to 400 mg/kg for durations varying from single treatment to periods including much of embryonic development. In the studies in which dosage was kept below the toxic level for the pregnant females malformed offspring rarely occurred, and at higher dosages only a low teratogenic potential was revealed. Results of rat studies with TCDD were variable. In two of three experiments very few malformed young occurred, but in the third an appreciable incidence of cleft palate was reported.

Mice proved to be more susceptible than the other species to the embryotoxic effects of both 2,4,5-T and TCDD. Both compounds produced low to moderate frequencies of cleft palate in all stocks tested but

they did not appear to be more teratogenic when given together than when given separately. Hamster studies with large doses of 2,4,5-T containing no detectable or low concentrations of dioxin (0.1 and 0.5 ppm) produced significant fetal mortality but relatively few instances (14/760 = 1.8%) of maldevelopment. Trials with high-dioxin-content 2,4,5-T (2.9 and 45 ppm) also caused appreciable fetal mortality but moderate frequencies of anomalies (16/209 = 7.6%).

Based on these data it can be concluded that: 1) doses of low-dioxin-content 2,4,5-T and of TCDD below the level producing maternal toxicity were without significant effect on prenatal development, producing little or no embryotoxicity in rats, rabbits, hamsters, sheep, and rhesus monkeys, and 2) these chemicals were more embryotoxic in mice, producing a low to moderate frequency of a specific malformation, cleft palate. The significance of the finding that TCDD in mice increased certain anomalies of the kidney, which occurred in low frequency in controls, can only be resolved by further investigation.

3. Human exposure during pregnancy. Reports have appeared in the news media that the use of 2,4,5-T was associated with an increased occurrence of congenital malformations and/or stillbirths in human beings in Vietnam; Globe, Arizona; and Sweden.

Vietnam. Because of the increased use of several defoliating chemicals by the United States Military in South Vietnam during the past several years, particular concern was aroused by the reports in Vietnamese newspapers between June 26 and July 5, 1969 of human birth defects attributed to these chemicals. Two surveys have been undertaken to evaluate the situation. One was conducted by Dr. R. T. Cutting, U. S.

Army Medical Research Team (Walter Reed Army Institute of Research), Dr. Tran Hun Phuoc, Ministry of Health, Government of the Republic of Vietnam, and three collaborators from the Military Assistance Command, Vietnam. A report was issued in December 1970 [24] and will be referred to here as the Army report. A second survey was recently made by the Herbicide Assessment Commission (HAC) of the American Association for the Advancement of Science, consisting of Drs. M. S. Meselson, A. H. Lesting and J. D. Constable [25][26]

The Army study surveyed obstetrical records mostly for the years 1960-69 of 22 provincial, district, and maternity hospitals in 18 cities and other areas in various geographical localities. In most hospitals the records consisted of daily summary ledgers, prepared by the chief midwives, and contained such relevant information as the age and parity of the mothers and the sex, weight, and general condition at birth of the babies. Space was provided for additional remarks concerning maternal or infant complications. In three hospitals such ledgers were not kept but instead individual records were available. In the hospital yielding the largest number of births, the Tu-Du Maternity Hospital in Saigon, as system of automatic data processing existed, which provided for separate recording of numerous categories of malformations.

Almost half a million births were included in cumulative records, and the overall recorded stillbirth and congenital malformation rates for the entire period were found to be 33.7 and 4.9 per 1000 livebirths, respectively. Attempts were made to analyze the information by geographical area, by year, and by intensity of herbicide spraying. The findings can be summarized as follows. (1) In four geographical regions - capital,

coastal, interior, and delta - the rates per 1000 livebirths of stillbirth and congenital malformation were 32.5 and 5.8, respectively, in the capital area, and 36.7 and 2.9 in the three remaining areas. The differences in these rates may be attributable to better maternal and neonatal care, or to more competent or thorough examination for congenital malformations in the capital area. (2) The rates for stillbirths declined and for congenital malformations remained unchanged during this 10-year period. (3) The only differences in these rates between the years 1960-65 and 1966-69, periods of relatively light and heavy defoliant spraying, respectively, was a downward trend (from 36.1 to 32.0 for stillbirths, and from 5.5 to 4.5 for congenital malformations). (4) There were no consistent differences between heavily and lightly defoliant-sprayed areas.

For the most part, however, the possibility of meaningful interpretations of the results of the Army study were precluded by their several limitations. First, it is obvious that only a fraction of the total births that occurred during these years were included in the records examined by the survey team. The report states that "RVN [Republic of Vietnam] officials estimate that currently only 70% of all births are reported to the MOH [Ministry of Health]" (emphasis added). The New York Times Encyclopedic Almanac for 1971 (p. 877) gives the estimated population of South Vietnam for 1970 at 18 million and the birth rate as 35-42 per 1000 population, which would yield between 630,000 and 750,000 births in 1970 [27]. The last complete year for which records were examined by the Army survey, 1969, yielded a total of 87,153 births.

Also the births that were included in the Army survey were far

from evenly distributed throughout the country, the three capital-area hospitals contributing over 67% of the total. In addition, the Chinese, who comprise a significant fraction of the population (over one million)[27/], were probably completely omitted since, as was stated by the Army report, they did not attend the hospitals surveyed. Finally, any hope of relating the results to variations in the degree or geographical region of herbicide spraying was frustrated by a number of factors. For example, changes in the local practices of referring difficult obstetrical cases to provincial hospitals, determined by availability of trained personnel in the centers, and increasing with gradual improvement in transportation and security, probably greatly influenced stillbirth and malformation rates in specific hospitals.

Probably most significant was the fact that populations most heavily exposed to 2,4,5-T were those most likely to be underrepresented in the Army survey or to be inadequately dealt with when recorded. Thus, as the HAC report stated, the bulk of 2,4,5-T used in Vietnam was sprayed in relatively remote and sparsely populated areas; the population directly exposed to 2,4,5-T probably did not exceed 5% and may have been 1% or less of the total population of Vietnam; and very likely a significant proportion of the exposed population consisted of Montagnard people, whose births usually did not occur in hospitals and rarely were included in medical records or statistics. It is equally probable that other remote and lightly populated areas were similarly underrepresented and incompletely recorded.

The Army survey noted that during 1960-69 there was a countrywide downward trend in the stillbirth rate. But, as was pointed out in the

HAC report, this was heavily influenced by data from the capital area, in which 67.8% of all the surveyed livebirths occurred, and which generally experienced little or no exposure to 2,4,5-T. Deducting the capital area data and considering only data from the other parts of the country apparently reverses the trend, giving stillbirth and malformation rates for 1960-65 (years of no or light spraying) and 1966-69 (years of heavy spraying) of 31.9 and 2.3, and 38.4 and 3.1, respectively. This would seem to indicate that in the remoter areas, where exposure could have been intense, stillbirth and malformation rates increased during years when spraying was heavy. A possible explanation for these apparent differences was provided by the HAC report, in noting that more complete recording and increased referral of difficult pregnancies from the countryside to the provincial hospitals occurred in these years. A more likely explanation, however, is that in recent years, as a larger and larger proportion of births was registered (e.g., number recorded in noncapital areas in 1960-65 was 37,951; in 1966-69, 113,358) a larger proportion of stillbirths was ascertained and a more complete examination for and/or recording of congenital malformations was made.

Particular attention was directed by the HAC reports to the records for the Tay Ninh Provincial Hospital, because although "the total number of directly exposed Vietnamese to 2,4,5-T is probably low, the northern portion of Tay Ninh has been heavily defoliated and the rivers draining the areas of defoliation run through the remainder of the province and are a source of fish for some of the population." Examining records that were apparently not available to the Army survey, the HAC found that

in 1968-69 the stillbirth rate recorded at the Tay Ninh City Provincial Hospital was 68.5, which they believed to be a higher rate than that found anywhere else by the Army survey. Although this is true, it should be noted that in two provincial hospitals, Qui Nhon, for which records only for 1966-69 were available, and Da Lat (nonpaying patients) during 1960-65, the stillbirth rates were not far below this, being 62.7 and 61.4, respectively.

The HAC also discovered how unreliable the records were regarding congenital malformations, since they noted that not a single malformation was recorded for the 2551 births in 1969 in the Tay Ninh Provincial Hospital, and on questioning the midwives it was learned that although a fair number of deformities had been seen none were reported. Another hospital, at Vung Tau, during most of 1968-70 reported no congenital malformations in 6198 births and a much lower stillbirth rate than did the Tay Ninh Hospital, yet it closely bordered and included in its referral area a zone of intense defoliation.

A further point needing critical scrutiny is the finding by the HAC of an apparent increased prevalence of children with spina bifida and isolated cleft palate among admissions to the Saigon Children's Hospital, the former increasing from 0.7% in 1959-66 to 2.1% in 1967-68, and the latter from 0.5% in 1959-65 to 2.6% in 1966-68. It should be emphasized that the figures do not pertain to incidence at birth, but to the percentage of malformed children admitted to this Hospital some time after birth for operative care. It should also be stated that at least 77.5% of all the admissions in 1959-69 came from Saigon or nearby areas in which defoliation was not practiced. Again the most likely explanation

of the apparent sudden rise in prevalence of these two malformations is more thorough examination and increased referral for surgical repair. Supporting this probability is the fact, noted by the Army Report, that the frequency of congenital malformations recorded in the capital area hospitals was much higher (although it varied greatly among the three hospitals) than in the remainder of the country, a fact in turn attributable to availability of more complete and competent medical services and personnel in the former than in the latter.

Summarizing the Vietnam data on human embryotoxicity, it can be said that (1) the sample of births surveyed was from year to year a variable but usually very small fraction of the total number, (2) it was quite unrepresentative of the geographic and ethnic distributions, (3) the heavily sprayed and otherwise exposed areas were greatly underrepresented, and (4) the birth records were not trustworthy and, therefore, the rates of stillbirth, and especially of congenital malformation, derived from them were equally unreliable. For example, the overall congenital malformation rate found in South Vietnam, 4.91 per 1000 live-births, is about half of what was reported in other studies in various parts of Asia [24], and possibly a quarter of what might actually exist at term. A further indication that the newborn children were not carefully examined is the absence of Down's syndrome in the list of specific malformations compiled by the Army survey, despite the fact that some Oriental populations have been reported to have an incidence of this condition not unlike that in Western populations [28].

Finally there is, and can be, no precise knowledge or reasonable approximation of the exposure to 2,4,5-T experienced by pregnant

Vietnamese women, including what amounts they ingested or absorbed and when this may have occurred during pregnancey. Thus, any attempt to relate birth defects or stillbirths to herbicide exposure is predestined to failure. It can only be concluded that the birth records that have been surveyed, and probably any that will be surveyed in the future, for South Vietnam for the period 1960-1970 cannot answer positively the questions about possible adverse prenatal effects following human exposure to 2,4,5-T. It must be emphasized, however, that the searches that have been made almost certainly would have revealed any marked increase in the incidence of birth defects or the introduction of a striking defect such as that produced by thalidomide. In spite of considerable effort, no such occurrences were found.

Globe, Arizona was another site of human exposure [29]. The herbicides used in the Kellner Canyon-Russell Gulch spray project near Globe were, in 1965 and 1966 the isooctyl esters of 2,4-D and 2,4,5-T, in 1968 an ester of Silvex (2,4,5-TP), and in 1969 about 97% Silvex (3680 lb) and almost 3% 2,4,5-T esters (Hercules Co., 30 gal.). The reports of harmful effects to animals and people from the spraying began during and after the 1969 spraying. Those concerning possible reproductive and embryonic effects consisted of two miscarriages by a woman, one in April and the other in December 1969; a number of stillbirths of kids; and one miscarriage in a goat. Two other alleged cases consisted of a deformed goat approximately 5 years old, and therefore born before any herbicide spraying in the area (incidentally the defects were not of developmental origin), and a chicken with a slipped tendon which was incubated 4 miles from the sprayed area and after the spraying occurred in 1969. In all likelihood none of these reported effects was due to the sprayings. Competent

medical and agricultural experts have been unable to find evidence of adverse effects on either human or animal reproduction that could be attributed to the defoliants applied during the Kellner Canyon-Russell Gulch spray project.

Swedish Lapland. Swedish government defoliation projects to improve the quality of forests in Lapland have been associated in the public press with the occurrence of human malformations and abortion among reindeer. The chlorophenoxy acids 2,4-D and 2,4,5-T were used in routine fashion for a number of years without reports of untoward effects until the spring of 1970 when several instances of unexplained death and abortion among reindeer were attributed to use of these compounds. A group of scientific experts has investigated these claims for the National Poisons and Pesticides Board [30/] and has failed to find a substantial basis for relating the toxic manifestations in these animals to ingestion of herbicides. Subsequently two instances of congenital malformations in human infants have been attributed to alleged exposure of pregnant women during application of the herbicides. Highly competent medical scientists at the Institute of Hygiene and the Teratological Laboratories of the Karolinska Institute of Stockholm and at the Institute of Human Genetics at Münster, Germany have been unable to find temporal or clinical evidence to suggest that the occurrence of these human birth defects was more than coincidentally related to defoliating operations in Sweden.

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GENERAL CONCLUSIONS

The Advisory Committee on 2,4,5-T has accepted as its primary objective the evaluation of hazards to human reproduction of continued use, under appropriate regulations, of the herbicide 2,4,5-T. Toward this end it has examined all available information pertinent to a scientific consideration of the subject.

The level of human exposure depends on rate of application of the herbicide, balanced against the rate at which it is removed from the environment. Current patterns of usage of 2,4,5-T and its known fate in various compartments of the environment, including the plant and animal foods of man, are such that any accumulation that might constitute a hazard to any aspect of human health is highly unlikely.

Special note has been taken of the toxic contaminant TCDD. The limited data now available indicate that this dioxin is not as rapidly degraded in the environment as is 2,4,5-T, but modern methods for the manufacture of the herbicide are capable of routinely producing a product with such a low level of contamination as to eliminate the likelihood of human toxicity from exposure to TCDD. Manufacturing standards must, however, be subject to continued monitoring.

Much of the general toxicity attributed to 2,4,5-T in the past now appears to have been caused by the contaminant TCDD. The herbicide when essentially free of this contaminant, e.g. 1 ppm, has relatively low toxicity for all animal forms in which it has been tested.

Particular attention was given to the teratogenic potential of

both 2,4,5-T and TCDD. Acceptable data are now available on the embryotoxicity of 2,4,5-T in 6 mammalian species, mouse, rat, hamster, rabbit, sheep and rhesus monkey. None of these showed adverse effects at dosage of 40 mg/kg/day of maternal weight.

The mouse appears to be more sensitive than the other forms studied in that it shows a low level of teratogenicity (cleft palate) at 100 mg/kg/day given throughout organogenesis, whereas hamster and rat required higher dosage to obtain comparable effects. It is likely that all species could be caused to show some embryotoxicity if 2,4,5-T dosage were raised high enough, a fact already well known for many prevalent environmental chemicals such as aspirin, caffein, nicotine and organic mercury.

The dioxin contaminant TCDD also has been shown to have a low teratogenic potential at doses in excess of 0.001 mg/kg, but this dosage level is virtually impossible with currently produced 2,4,5-T. No evidence has been found of significant potentiative interaction between 2,4,5-T and TCDD.

No evidence has been found of adverse effects on human reproduction in three separate locations, n[illegible] Vietnam; Globe, Arizona; and Sweden, where pregnant women have al[illegible] been exposed to high levels of 2,4,5-T.

On the basis of these observations, it is concluded that, as presently produced and as applied according to regulations in force prior to April 1970, 2,4,5-T represents no hazard to human reproduction.

RECOMMENDATIONS

The Advisory Committee on 2,4,5-T after careful consideration of available information on potential hazards to man, particularly as regards reproductive functions, of continued, regulated use of 2,4,5-T, recommends the following:

1. That registration for use of 2,4,5-trichlorophenoxyacetic acid and its esters be restored to the status existing prior to April 1970, with the following exceptions.

2. That certain specific limitations and qualifications be added to the previously existing registration, as follows:

a. A permissible residue or not more than 0.1 ppm of 2,4,5-T on the edible parts of food products and in potable water for human consumption be accepted. It is recognized that very few foods tested to date have contained this level of residue, but it is probable that some of the reports of no residue in the past were due to limited sensitivity of the analytical method. In view of recent and future advances in methodology, which tend to make zero residues of anything increasingly unlikely, a more realistic policy would be the setting of safe tolerance limits at this time.

b. A limit of 0.5 ppm of contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin be set for existing inventories of 2,4,5-T, except as specified in item c. below, and a limit of 0.1 ppm of contamination with this dioxin be established in all future production of 2,4,5-T. Surveillance should be maintained by requiring that a manufacturer submit a reference sample and a certified analysis of each future production lot to the Environmental Protection Agency.

c. All formulations to be used around the home and in recreational areas as of present date should be limited to 0.1 ppm of the dioxin, TCDD, and also should bear a conspicuous warning, e.g., "This compound may be dangerous to pregnant women and animals and its use must be such as to reduce the possibility of exposure to an absolute minimum".

3. That existing deficiencies in information relative to possible accumulation in the soil and possible magnification in the food chain of the dioxin TCDD be rectified by specific research directed to this end, with these questions to be subjected to scientific review within three years of the present date and yearly thereafter until these questions are resolved.

4. That additional post-registration monitoring for adverse effects of agricultural chemicals be established, to include both surveillance for such effects in man and domestic and wild animals, as well as consideration of the applicability of new methodology that may be evolved for specialized testing, e.g., for carcinogenesis, mutagenesis or teratogenesis.

Date: May 7, 1971

Respectfully submitted,

James G. Wilson

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Objections to and Modification of the Final Report and Recommendations of the 2,4,5-T Advisory Committee

The report by the 2,4,5-T Advisory Committee is basically an accurate statement of the present state of information; however, it falls short of being completely fair in its evaluation of the evidence on which conclusions are based. It is true that considerable uncertainty exists about the teratogenic potential of 2,4,5-T (and of its impurities) especially for the small doses at which man may make effective contact with this substance. On the other hand, the report is overoptimistic in assessing the implications of data so that it may well underestimate what dangers may lurk in the unrestricted use of 2,4,5-T. Particularly:

The data do not necessarily justify a conclusion that there is a level at which TCDD is not teratogenic.

There is an unjustified certainty that Vietnam birth records do not show teratogenic effects.

The report fails to consider the consequences of the (admitted) uncertainty about the fate of TCDD in the food chain and in tissue.

The report does not weigh risk vs. benefits, as it was charged to do.

The report presumes to lecture the scientific community on the wisdom of instituting a "permissible residue" of substances thought to be teratogenic.

The report is overoptimistic in believing that recommendations for needed research will be followed by industry or by public agencies once a decision has been rendered to restore 2,4,5-T to unrestricted use.

We can only conclude that the Surgeon General was justified in feeling that a prudent course of action must be based on the decision that exposure to this herbicide may present an imminent hazard to women of childbearing age. Hence, we can only recommend that the registration of 2,4,5-T be suspended and/or cancelled for use around the home, recreation areas, and similar sites and on all crops intended for human consumption. However, the use of 2,4,5-T may be permitted under certain conditions for uses in forestration and rights-of-way providing:

1. That the limit be set of 0.1 ppm of contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin for all future production of 2,4,5-T. (However, the use of present inventories may be permitted until used up providing the amount of contaminant in them does not exceed .5 ppm of the dioxin TCDD.)
2. That 2,4,5-T be applied no more often than once a year at any one site.
3. That 2,4,5-T be applied with proper caution so that it will not contaminate other areas where it may come into human contact.

We also recommend that this action be reviewed again when the existing deficiencies in information relative to possible accumulation in the soil and possible magnification in the food chain of the dioxin TCDD have been rectified by specific research directed toward that end.

It is always difficult to make decisions in the face of uncertainty. The insufficient data makes the work of the committee very difficult. The fact that ours is the view of the minority ought to strengthen the impression that the committee labored honestly and conscientiously to deduce the best recommendations from a confused aggregate of observations.

Theodor D. Sterling

5/5/71

Additional Comments

It Is Not Quite Certain at What Dose TCDD Has No Effect

The report gives the impression that 2,4,5-T shows a teratogenic effect uniformly at high doses only. One reason for that impression is that most investigations concentrate on doses of 100 mg/kg or more so that data are lacking to a large extent on how much of teratogenic effects could show up at smaller doses. Yet, there are a number of studies that do show definite effects for doses of less than 100 mg/kg of weight.* Also, the dose effect of 2,4,5-T depends largely on its impurities, especially on TCDD. The experiments which provide the basic animal data and the analysis of these data unfortunately were not done with the sophistication necessary to throw light on the effect of 2,4,5-T and TCDD at very low doses. Many of the reports presented no more than tables of group means, and some even presented pages and pages of undigested numbers on individual observations. It is difficult to draw any final and firm conclusion from data such as these. Nevertheless, there are sufficient instances where teratogenic

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* For example, on rabbits increased resorption and diminished fetal weight reported by Emerson, J. L., Thompson, D. J., Gerbig, C. G., and Robinson, V. B.: Teratogenic Study of 2,4,5-Trichlorophenoxyacetic Acid in the Rabbit, The Dow Chemical Co., Zionsville, Indiana.

A number of instances are cited by Epstein, S. S., of the Children's Cancer Research Foundation, Inc. and Harvard Medical School, Boston, Mass., 4/14/70, Subject: Teratogenic effects of 2,4,5-T formulations.

Another example is the study on hamsters by Courtney, K. D., Moore, J. A., Gaylor, D. W., Hogen, M. D., Falk, H. L.: Summary Teratogen Study NIEHS.

properties have been observed at lower doses than 100 mg/kg so that the question whether there is a zero effect for some dose is not easily answered. A case in point is the data included in this report by the chairman of the committee. The experiment provides for doses of 100, 200, and 400 mg/kg but only tests at one single low dose, that of 20 mg/kg, although the effect of a dose at this low level is of utmost importance. Also, there are no control animals. Cumulative experience with untreated controls over the past four years and cumulative vehicle treated controls (per gavage) over the past four years are used as controls. Given our knowledge of variation in experiments, it is difficult to understand why this important experiment was performed without a concurrent control. Yet, per cent dead or resorbed fetuses show a trend toward lower dose which is still detectable at 20 mg/kg. (Whether or not we think of this effect as large or small will depend on whether or not we are willing to accept Control 1. or Control 2. of that study.) Taking these factors into consideration, it becomes difficult to see how the report can conclude that "doses of low-dioxin-content 2,4,5-T and of TCDD below the level producing maternal toxicity were without significant effect on prenatal development producing little or no embryo-toxicity in rats, rabbits, hamsters, sheep, and rhesus monkeys" (page 50, Final Draft). If anything, the conclusion ought to have read that, despite the small amount of data present, some of it does point to teratogenicity at lower doses.

The Uncertainty About the Vietnam Human Data Does Not Mean These Data Show No Effects of 2,4,5-T on Stillbirth and Malformation

2,4,5-T was used extensively in Vietnam for defoliation. Unfortunately, birth records show a confused and confusing picture of what happened to malformation in Vietnam during the years in which defoliation efforts increased

and how stillbirth rates compare between regions that are heavily defoliated and regions that are not. The Army survey notes that during 1966 to 1969 there was a countrywide downward trend in the stillbirth rate. But the HAC report points out that this conclusion was heavily influenced by data from the capitol area in which 67.8 per cent of all live births surveyed occurred and which, in addition, generally experienced little or no exposure to 2,4,5-T. Deducting the capitol area data and considering only that from other parts of the country, reverses the trend and results in lower stillbirth and malformation rates for 1960-65 (years of no or light spray) than for 1966-69 (years of heavy spraying). Also, HAC found that the 1968-69 stillbirth rates recorded at Tay Ninh City Provincial Hospital, a hospital which was in a region heavily defoliated and through which rivers draining areas of defoliation run, had a recorded stillbirth rate of 68.5, which they believe to be higher than that found anywhere else.

The 2,4,5-T Advisory Committee report goes into the unreliability of all the human data that comes from Vietnam in great detail. It is true that the instances cited might easily have created a spurious impression of an increased stillbirth rate for the defoliated regions or periods. However, the opposite might be just as true. Factors could just as easily have worked to hide a large stillbirth rate than to spuriously create one. If we already speculate, we might just as easily speculate the other way. Thus, the best we can say is that these data do not definitely show an effect of defoliation. However, they do show an effect which has to be <u>explained away</u>. It is up to the committee to register our doubts, but it is unseemly to spend page after page denying the reality of the Vietnam observation in the face of the careful report by a select committee of the American Association for the Advancement of Science.

The Report Fails to Consider the Uncertainty About the Fate of TCDD

It is not possible to produce 2,4,5-T without impurities, especially the dioxin TCDD. These impurities have been shown to be toxic and teratogenic in the extreme. (In fact, the recommendations of the report to restrict the permissible level of TCDD in 2,4,5-T and to monitor this restriction is in recognition of its hazard.)

While 2,4,5-T is quickly eliminated from the soil by biological and other actions, the same cannot be said of TCDD. The report notes that TCDD might accumulate in the soil from one year to the next. There is also evidence that a small amount of the soil's TCDD is absorbed by plants. But there is no information on the concentration of TCDD in the food chain at this level, nor is there information as to what extent TCDD may be stored by animal tissues. Again, the report speculates that TCDD is not stored to a significant extent because it is not easily soluble in oils. However, may it not be stored by other tissues besides fat, and, in fact, may it not be stored more by animal fatty cells than in oils? (There is some evidence that TCDD, when digested, finds its way to every tissue in the body.)

There is no question but that some doubts exist in the minds of the committee on this point. The recommendations acknowledge these doubts and ask that the deficiency in information relative to possible accumulation in the soil and possible magnification in the food chain of the dioxin TCDD be rectified by specific research directed to this end, with this question to be subjected to scientific review within three years of the present date and yearly thereafter until these questions are resolved (Section 3 of Final Draft Recommendations).

We find it difficult to go along with this reasoning. After recent experience with DDT and with mercury, it would be reckless to leave such questions in abeyance while approving the unrestricted use of 2,4,5-T. Nothing would be lost by waiting two or three years while this question can be settled. On the other hand, a great deal of damage may be created if the committee restores 2,4,5-T to its normal use while hoping that further research will justify our confidence in having made a correct guess. Moreover, the restriction of 2,4,5-T from regular use would work as a powerful motive, spurring industry and concerned government agencies to seek to settle this important question by initiating appropriate studies. Yet, at the same time, the use of 2,4,5-T could be maintained in all those instances where contamination of food or people would be unlikely.

Considering the Risk/Benefit Equation

A curious situation emerges in evaluating the benefit of 2,4,5-T. It is apparently of major value in its nonfarm uses, especially for forestry and road clearance, where it can be applied sparingly and with a great deal of expertise. Also, in forestration 2,4,5-T may be reapplied only every few years. The other uses in or near food crops and around the home represent less frequent areas of application but may expose very large numbers of individuals to 2,4,5-T, to its impurities, and to its residues. Thus, it is not true that all uses of 2,4,5-T are equivalently beneficial.

Many of the nonfarm uses of 2,4,5-T clearly have national benefit. On the other hand, the use of 2,4,5-T for the growing of crops, especially rice, is basically of benefit to a few farm industries because it increases the yield per acre of cultivated ground. Since there are available many acres that are not now cultivated, there does not appear to be a national

urgency to support that limited use of 2,4,5-T until important questions about buildup of TCDD have been settled. Similarly, the use of 2,4,5-T for grazing land is of relatively small benefit for a small number of people. Finally, although the use of 2,4,5-T around the house may be thought of as of national importance, adequate alternate means for home gardening do exist, so that it may be best to avoid for the moment the possible risk of direct contact with man.

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5/5/71

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